INFECTION AND IMMUNITY, Jan. 1984, p. 440–441
0019-9567/84/010440-02$02.00/0

Vol. 43, No. 1

Experimental *Klebsiella pneumoniae* Burn Wound Sepsis: Role of Capsular Polysaccharide

S. J. CRYZ, JR.,* E. FÜRER, AND R. GERMANIER
Swiss Serum and Vaccine Institute, Berne, Switzerland

Received 22 July 1983/Accepted 13 October 1983

The role of *Klebsiella pneumoniae* capsular polysaccharide in relation to virulence in a murine burn wound sepsis model was investigated. Burn trauma markedly predisposed mice to lethal *K. pneumoniae* sepsis. A highly encapsulated variant (KP1-O) derived from *K. pneumoniae* KP1 was found to be extremely virulent for burned mice (50% lethal dose < 10 organisms), whereas another variant (KP1-T), which possessed a much smaller capsule, was comparatively nonvirulent (50% lethal dose > 10^6 organisms). Production of large quantities of capsular material by KP1-O allowed for its rapid growth in vivo and persistence in the blood and liver. These traits were not demonstrated by KP1-T, which was effectively cleared after challenge.

Nosocomial infections due to *Klebsiella* species are a major cause of morbidity and mortality among several patient populations (5, 9). Of these groups, burn patients are particularly susceptible to life-threatening infections (8, 10, 13). In two reports, *Klebsiella* was second only to *Pseudomonas aeruginosa* as the cause of the bacteremia in burn patients (8, 10). Although several excellent experimental animal models exist for the study of pulmonary infections due to *Klebsiella* (2, 4), attempts to induce fatal burn wound sepsis have met with little success (15). Recent findings by Domenico et al. (4) demonstrating that the virulence of *Klebsiella pneumoniae*, both in a pulmonary and in an intraperitoneal challenge model, is greatly influenced by capsule size have led us to investigate this aspect in relationship to virulence in a murine burn model.

Two variants derived from *K. pneumoniae* KP1 (ATCC 8047, American Type Culture Collection, Rockville, Md.) were employed: KP1-O, which possesses a large capsule, and KP1-T, whose capsule is markedly smaller (approximately one-third that of KP1-O) were obtained from D. Straus, Texas Tech University Health Science Center, Lubbock, Tex. (4). Cultures were routinely grown on tryptic soy broth with dextrose (Difco Laboratories, Detroit, Mich.) at 37°C with shaking. The murine burn wound model of Stieritz and Holder (15) was used with slight modifications. The challenge organisms were grown to mid-log phase (absorbance at 540 nm = 0.3) and diluted in phosphate-buffered saline. Mice (18 to 20 g, Swiss-Webster white) were anesthetized with methoxylflurane (Penthrane, Abbott Laboratories, North Chicago, Ill.). Immediately after a 10-s ethanol burn over a 2-cm^2 area of the back, the bacterial challenge was given subcutaneously in 0.5 ml of phosphate-buffered saline. Mortality was recorded for 10 days postchallenge. The mean lethal dose (LD_{50}) was calculated by the method of Reed and Muench (14). Groups of six mice were used for each dilution. Quantitation of bacteria in the blood and in tissues was performed as previously described (3).

The effect of prior burn trauma on the virulence of *K. pneumoniae* was studied first. The LD_{50} of KP1-O (highly encapsulated) for burned and for normal mice was determined (Table 1). KP1-O was found to be extremely virulent in burned mice with an LD_{50} < 10 bacteria. However, it was comparatively nonvirulent for normal mice, as shown by a greater than 10,000-fold difference in the LD_{50} value between the two groups. Therefore, burn trauma markedly predisposes mice to fatal infection with *K. pneumoniae*.

Next, the role that capsule size plays in the virulence of *K. pneumoniae* for burned mice was investigated by comparing the LD_{50} values for KP1-O and KP1-T (Table 2). As in the previous experiment, the LD_{50} for KP1-O was <10 bacteria. KP1-T was found to be far less virulent, with LD_{50} values on the order of six magnitudes higher than KP1-O in both experiments. These findings indicate that the amount of capsular material produced by *K. pneumoniae* plays a critical role in virulence.

Bacterial growth and dissemination after challenge with KP1-O or KP1-T was monitored in an effort to better characterize the infectious process (Fig. 1). KP1-O proliferated rapidly in burned skin, with bacterial numbers exceeding 10^9 per gram of skin by 48 h postchallenge. The bacterial load in the skin remained at this level throughout the course of the experiment. Bacteremia and liver colonization were first seen at 48 h postinfection. Deaths due to KP1-O sepsis occurred on days 3 through 5. In contrast, KP1-T displayed only limited multiplication in the skin, being cleared by day 3. Liver colonization and bacteremia were not observed. Therefore, possession of a large capsule allowed not only for sustained bacterial growth in the skin, but also for subsequent invasion of the bloodstream and organ colonization.

The present study demonstrates that the ability of *K. pneumoniae* to cause lethal burn wound sepsis in mice is dependent upon the amount of capsular polysaccharide produced. This is in agreement with previous studies using pulmonary or intraperitoneal challenge models (4, 6). Several hypotheses exist as to how *K. pneumoniae* capsular polysaccharide acts to increase virulence: (i) antiphagocytic activity (6, 16); (ii) induction of immune paralysis (1, 11); and

TABLE 1. Effect of burn trauma on the virulence of *K. pneumoniae* KP1-O

Burn trauma induced	LD_{50}[a]	MTD (h)[b]
Yes	$<0.72 \times 10^1$	158 ± 10.7
No	3.96×10^5	136 ± 24.5

[a] Expressed as the number of viable infecting cells.
[b] Mean time to death ± standard deviation.

* Corresponding author.

TABLE 2. Virulence of *K. pneumoniae* KP1-O and KP1-T for burned mice

Challenge strain	Expt 1		Expt 2	
	LD_{50}	MTD ± SD[a] (h)	LD_{50}	MTD ± SD[a] (h)
KP1-O	$<0.75 \times 10^{1}$[b]	144 ± 33	$<0.65 \times 10^{1}$[b]	180 ± 10.7
KP1-T	$>3 \times 10^{6}$[c]		1.12×10^{7}	96

[a] Mean time to death ± standard deviation.
[b] Lowest challenge dose tested. Less than 50% of animals survived.
[c] Highest challenge dose tested. All animals survived.

(iii) providing a barrier against nonspecific host defense systems (4). Which mechanism or mechanisms functioned in the animal model employed in the current study remains to be determined.

The animal model used in the current study was initially developed to investigate the pathogenesis of *P. aeruginosa* burn wound sepsis (15). The LD_{50} for *K. pneumoniae* KP1-O was comparable to that seen for highly virulent strains of *P. aeruginosa* (<10 bacteria) (15). However, infection with *P. aeruginosa* is much more rapidly fatal compared with infection with KP1-O (15). Whereas the mean time to death with *P. aeruginosa* is approximately 30 h, that for KP1-O was found to be 150 h. This difference is most likely due to the production of extracellular enzymes (proteases and toxins) by *P. aeruginosa* which enhance bacterial growth in burned skin, allowing for invasion of the bloodstream (7, 12). Production of similar virulence factors by *K. pneumoniae* has not been reported. The use of a murine burn wound model with highly virulent challenge strains of *K. pneumoniae* may prove to be useful in evaluation of immunoprophylactic/immunotherapeutic agents for the prevention of fatal sepsis.

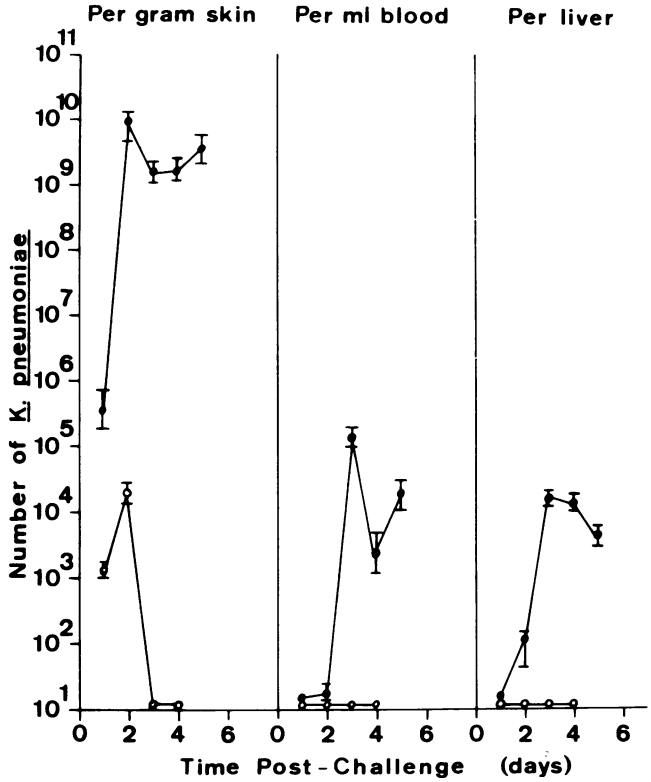

FIG. 1. Quantitation of bacteria at various times after challenge of burned mice with approximately 10^2 organisms. Time 0 = time of challenge. Each time point represents the mean for three mice ± the standard error. Symbols: (●) KP1-O; (○) KP1-T.

Excellent technical assistance was provided by E. Zaugg. We thank V. Rupp for help in preparation of this manuscript.

LITERATURE CITED

1. **Batshon, B. A., H. Baer, and M. F. Schaffer.** 1963. Immunological paralysis in mice by *Klebsiella pneumoniae* type 2 polysaccharide. J. Immunol. **90**:121–126.
2. **Berendt, R. F., G. L. Knutsen, and M. C. Powanda.** 1978. Nonhuman primate model for the study of respiratory *Klebsiella pneumoniae* infection. Infect. Immun. **22**:275–281.
3. **Cryz, S. J., Jr., E. Fürer, and R. Germanier.** 1982. Simple model for the study of *Pseudomonas aeruginosa* infections in leukopenic mice. Infect. Immun. **39**:1067–1071.
4. **Domenico, P., W. G. Johanson, Jr., and D. C. Straus.** 1982. Lobar pneumonia in rats produced by clinical isolates of *Klebsiella pneumoniae*. Infect. Immun. **37**:327–335.
5. **Dupont, H. L., and W. W. Spink.** 1969. Infections due to gram-negative organisms: an analysis of 860 patients with bacteremia at the University of Minnesota Medical Center, 1958–1966. Medicine **48**:307–332.
6. **Ehrenworth, L., and H. Baer.** 1956. The pathogenicity of *Klebsiella pneumoniae* for mice: the relationship to the quantity and rate of production of type-specific capsular polysaccharide. J. Bacteriol. **72**:713–717.
7. **Holder, I. A., and C. G. Haidaris.** 1979. Experimental studies of the pathogenesis of infections due to *Pseudomonas aeruginosa*: extracellular protease and elastase as *in vivo* virulence factors. Can. J. Microbiol. **25**:593–599.
8. **Jones, R. J., E. A. Roe, and J. L. Gupta.** 1979. Controlled trials on a polyvalent pseudomonas vaccine in burns. Lancet **i**:977–983.
9. **Montgomerie, J. Z.** 1979. Epidemiology of *Klebsiella* and hospitalassociated infections. Rev. Infect. Dis. **1**:736–753.
10. **Nathan, P., I. A. Holder, and B. G. Mac Millan.** 1973. Burn wounds: microbiology, local host defenses, and current therapy. Crit. Rev. Clin. Lab. Sci. **4**:61–100.
11. **Ørskov, I.** 1956. "Immunological paralysis" induced in rabbits by a heavily capsulated Klebsiella strain. Acta. Pathol. Microbiol. Scand. **38**:375–384.
12. **Pavlovskis, O. R., B. H. Iglewski, and M. Pollack.** 1978. Mechanism of action of *Pseudomonas aeruginosa* exotoxin A in experimental mouse infections: adenosine diphosphate ribosylation of elongation factor 2. Infect. Immun. **19**:29–33.
13. **Pruitt, B. A., Jr.** 1974. Infections caused by *Pseudomonas* species in patients with burns and in other surgical patients. J. Infect. Dis. **130**(Suppl.):8–13.
14. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. **27**:493–497.
15. **Stieritz, D. D., and I. A. Holder.** 1975. Experimental studies of the pathogenesis of infections due to *Pseudomonas aeruginosa*: description of a burned mouse model. J. Infect. Dis. **131**:688–691.
16. **Yokochi, T., I. Nakashima, and N. Kato.** 1977. Effect of capsular polysaccharide of *Klebsiella pneumoniae* on the differentiation and functional capacity of macrophages cultured *in vitro*. Microbiol. Immunol. **21**:601–610.